generated individual with a new universal counterpart soul and a new individual counterpart soul. But in spite of the new life, the new being he had become, the transmutations preserved a link with his past, with his previous existence, his origin in ancestry, and his origin in deity.

As to the metaphoric Shaman language, the point is that it is used in the unconscious state, when the Shaman is in a trance, etc., as well as in the conscious state, in the entire oral traditional literature of the old Eskimo culture.

Until now very little has been recorded of the Shaman language, and its significance and meaning have not been understood. The Shaman metaphoric words have been translated as ordinary words, when they are something entirely different. They are expressions of his soul, a part of himself, expressions too of the soul of his fellow Eskimos—a part of themselves. The myths, the legends, the entire "literature" of the aboriginal Eskimo are interwoven with Shaman words. This has definitely not been known before. There is much, much more to be recorded. We must study this "literature" in an entirely new light, and it must be done now, while some of the older Eskimos who possess this unique knowledge are still alive. In a few more years it will be too late. The only effective way of doing this is to have an Institute of Eskimology, which for a number of years I have been trying to organize. Such an Institute, which could be established at a modest cost, could accomplish this urgent task while there is still time.

Photocontrol of Anthocyanin Synthesis

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We have no way of knowing when man began to appreciate the beauty of the autumn coloration that appears in the deciduous forests of the temperate and subarctic zones. Nor do we know when he began to use and cultivate flowers for ornamental purposes. We are not even certain when man began to correlate fruit ripeness with color. As they became aware of the plants around them people must have noticed the predominance of reds and blues and attempts must have been made to use these colors as dyes for religious costumes, face and body painting, etc. Thus, the fact that the substances responsible for the blue and red colors of many plant parts are water-soluble probably was apparent at an early time. Because they were soluble in water the colored materials were easily separated from the plant tissue and, of course, had to be given a name. Undoubtedly many names were given to these water-soluble, colored substances from plants, but the one we use today is anthocyanin, from the Greek *anthos*, a flower, plus *kyanos*, dark blue.

We know that man began inquiring into the nature of anthocyanin over 300 years ago. In 1664, for example, Robert Boyle (1) noted that an extract from blue-violet petals turned red when an acid such as vinegar was added to the solution. By 1800 (2) it was known that light was generally required for anthocyanin synthesis, and by 1900 (3) the evidence clearly showed that the accumulation of soluble carbohydrates was essential.

The chemical identity of anthocyanin

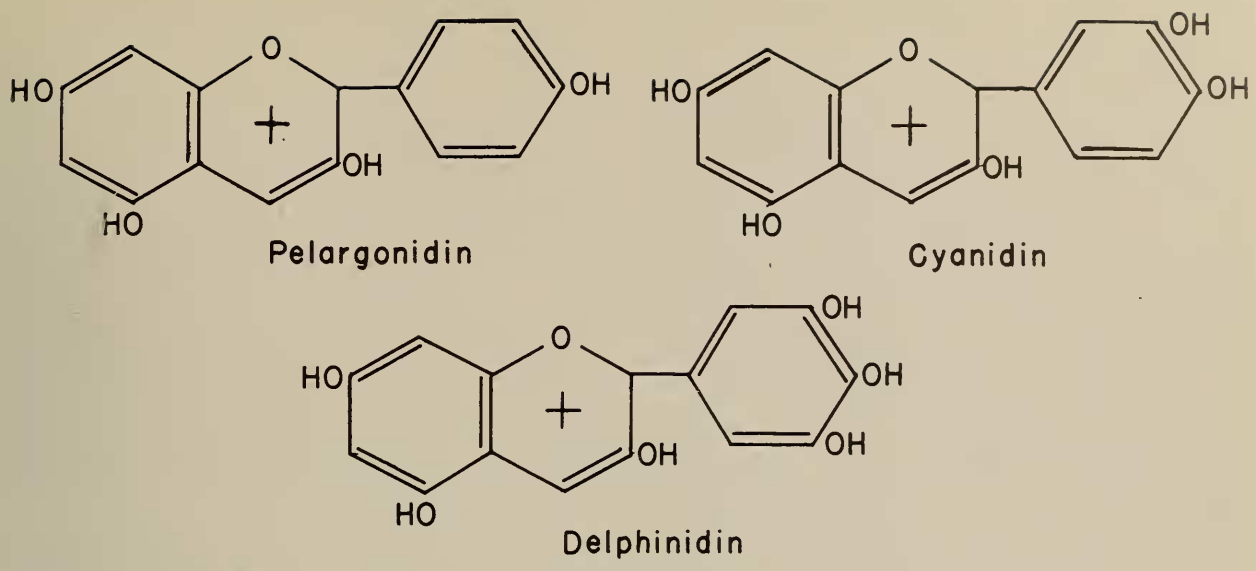

Fig.1. The three most common anthocyanidins.

was established in 1913 by Richard Willstätter (4). A number of other investigators added details that have resulted in a rather complete picture of the chemical structure. Anthocyanins are glycosides that are hydrolyzed on heating with acid into sugars and an anthocyanidin. The anthocyanin glycoside is frequently formed by replacing the hydroxyl group of the middle ring by sugars. Sugars can also be attached to places other than the central ring but normally only in the 3 position or in the 3 and 5 positions. Identification of an unknown anthocyanin thus depends on identifying the anthocyanidin and determining the number and kind of sugars present and where they are attached. Since anthocyanins are sometimes formed as acylated glycosides, the presence of an acyl component must be determined and the associated organic acid identified.

As might be expected, a large number of anthocyanins can be formed from only a few anthocyanidins. For many years the major anthocyanidins were pelargonidin, cyanidin, and delphinidin, and most of the anthocyanins were placed in one of these three categories (Fig. 1). By 1958 (5), 10 anthocyanidins could be listed, and what were once considered as single anthocyanins now proved to be several, and in some cases not anthocyanins at all. For example, what had been considered to be a cyanidin glycoside in *Spirodela oligorrhiza* was reinvestigated by chromatographic means and shown not to be directly related to any known anthocyanin (6).

The distribution of the anthocyanins in flower parts is often complex, and this complexity has yielded results of taxonomic interest. In Papaver species, for example, the species can be determined by the distribution of the six anthocyanins in the flowers (7).

A number of factors influence the formation of anthocyanin. Genetics is, of course, of prime consideration. Texas milo seedlings, for example, produce twice as much anthocyanin under a given set of conditions as do seedlings of Texas Dwarf white milo. An accumulation of soluble carbohydrates is a definite requirement for anthocyanin synthesis and any condition that affects this accumulation also affects the amount of anthocyanin produced. Thus, in nature cool temperatures would operate to reduce respiration, thereby allowing an accumulation of sugars and a corresponding production of anthocyanin.

Light exerts a most emphatic control over anthocyanin synthesis, and it is the photocontrol of anthocyanin that we wish to consider here. When all other conditions are optimum, anthocyanin will usually not be formed in the absence of light. In the few instances where some an-

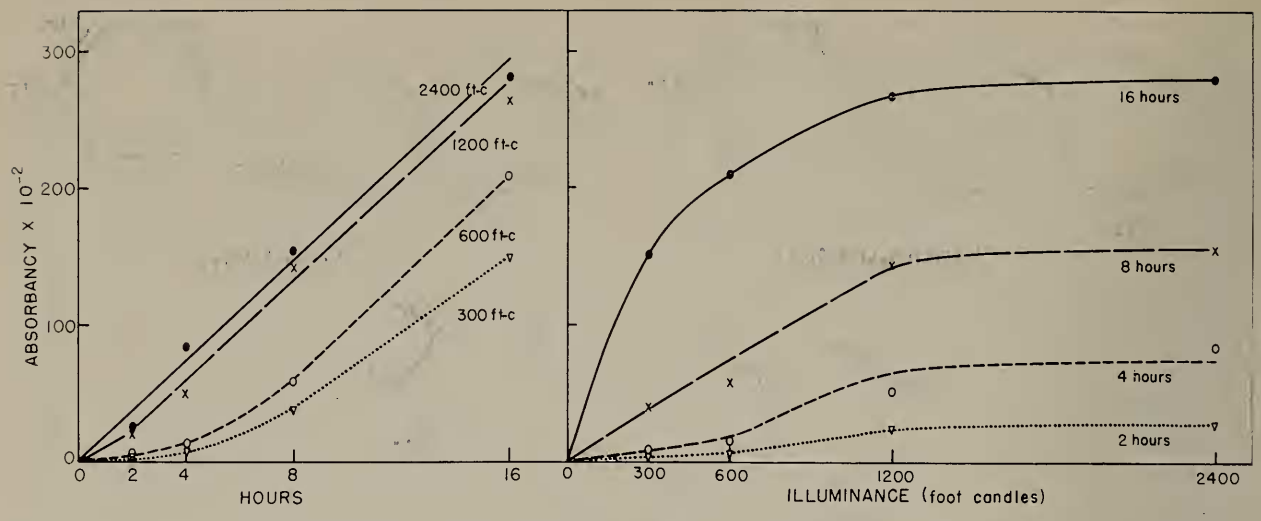

Fig. 2. Amount of anthocyanin formed in response to various durations of light from fluorescent lamps at several illumination levels. Measurement was made after a 24-hour dark incubation period (left). Amount of anthocyanin formed in response to various illumination levels after several durations of exposure to light from fluorescent lamps. Measurements were made after a 24-hour dark incubation period (right).

thocyanin is produced in darkness, the amount is increased many fold when the plant material is illuminated. Since light can be introduced into a biological system with a minimum of disturbance to the cellular processes, it provides a unique tool for studies of the overall system of synthesis. Conversely, anthocyanin production provides an excellent physiological system with which to study the photoreactions involved.

Although anthocyanin appears in a large number of plants, its production has been investigated in detail (8, 9, 10, 11, 12) in only a few. We will confine our discussion to milo (11), turnip, and red cabbage seedlings (8), and to the skin of apple fruits (10).

Table 1. Formation of anthocyanin after exposures to 1,600 ft-c fluorescent light in several varieties of Sorghum vulgare *(milo, kaffir, and sorghum)*

Variety	*Duration of exposure (hrs)*	*A x 10^{-2}*
Wheatland	16	280
Sumac	16	256
Hegari	16	140
Leoti	16	34
Sapling	16	17
Planter	23	235
Chinese Amber	23	196
Dwarf Ashburn	23	175
Texas milo	23	134
Texas DW milo	23	77
Red Kaffir	23	32
Feterita	23	10

Milo seedlings grown in the dark do not produce any anthocyanin. If dark-grown seedlings three and one-half to four days old are placed in the light, they become a faint pink in about six hours. If the seedlings are placed in darkness for 20 to 24 hours after the light period, the elongate first internode becomes an intense red. An examination of the seedlings shows that the root, the coleoptile, and the rudimentary leaves are not necessary for the formation of anthocyanin in the first internode. However, the seed should remain attached to the shoot if an appreciable amount of anthocyanin is to be formed. If the shoot is removed and the seed left attached to the root, the root will form anthocyanin in the presence of light.

A large number of varieties of *Sorghum vulgare* form anthocyanin when the dark-grown seedling is exposed to light (Table 1). This discussion of milo will deal only with the responses of the variety Wheatland. The amount of anthocyanin formed by Wheatland milo seedlings is dependent upon the light intensity and the duration

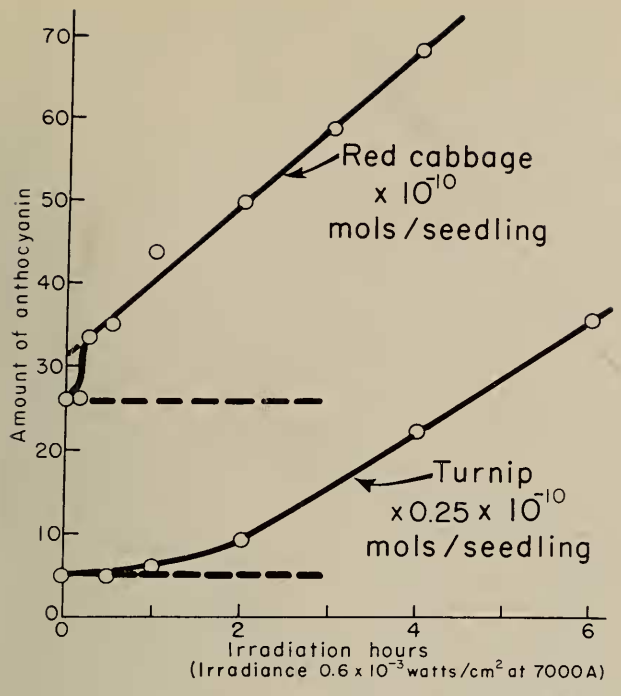

Fig. 3. Variation in anthocyanin synthesis in red cabbage and turnip seedlings with time of irradiation at a constant irradiance equivalent in photochemical effectiveness to 0.6 x 10^{-3} watts/cm^2 at 7000 A. The seedlings were extracted for analysis 24 hours after the beginning of irradiation. Dashed lines indicate synthesis in unirradiated seedlings (8).

of exposure. However, double the intensity and half the time does not induce the same result as unit intensity and time. For example, doubling the illuminance from 1,200 to 2,400 ft-c does not increase the amount of anthocyanin appreciably, but doubling the time of exposure at 1,200 ft-c from 8 to 16 hours approximately doubles the amount of anthocyanin (Fig. 2).

At moderately high light intensities milo seedlings accumulate anthocyanin at a constant rate for at least the first 16 hours of irradiation. At lower intensities, however, a lag period of four to six hours occurs before the linear phase of anthocyanin synthesis begins. Light given continuously over a certain period is not utilized as efficiently as is light given in cycles. Light in cycles of 2 minutes light, 18 minutes dark over a four-hour period, for example, was used much more efficiently than it was in four hours of continuous light (Table 2).

The amount of anthocyanin formed by turnip and red cabbage seedlings is linearly dependent upon the duration of exposure to light (Fig. 3). Anthocyanin synthesis also depends on the intensity of the light, but intensity is not so important as time. As in milo, the reciprocity law fails in turnip and red cabbage. The time course for anthocyanin synthesis in turnip seedlings shows a time lag prior to the linear phase, whereas in red cabbage and milo it does not. Perhaps the time lag would disappear at higher energies, but they were not available at the time the experiments were conducted. Red cabbage differs from milo and turnip seedlings in that it makes an appreciable amount of anthocyanin in complete darkness.

The red color in apples requires light for its formation and the color variation of different kinds of apples indicates different abilities to synthesize anthocyanin (Fig. 4). Apple skin peeled from the apple fruit forms anthocyanin as well as it does on the fruit, providing the pieces of skin are floated on a sugar solution. Since the apple skin does not grow, it is a simpler total system than seedlings and therefore merits attention. Apples picked green were peeled and the green peel cut into 1-cm^2 sections. The sections were floated on 0.3 *M* sucrose and exposed to various

Table 2. Relative accumulation of anthocyanin per unit of light (11)*

Light per cycle (min)	*Relative accumulation per minute of light* (A x 10^{-2})
2	11.0
4	8.0
6	6.7
8	5.4
10	4.0
12	3.8
14	3.4
16	3.1
18	3.0
20	3.2

* One minute in each 20-minute cycle at an illuminance of 2,400 ft-c from fluorescent lamps. Total time was 4 hours; total light was 12 minutes.

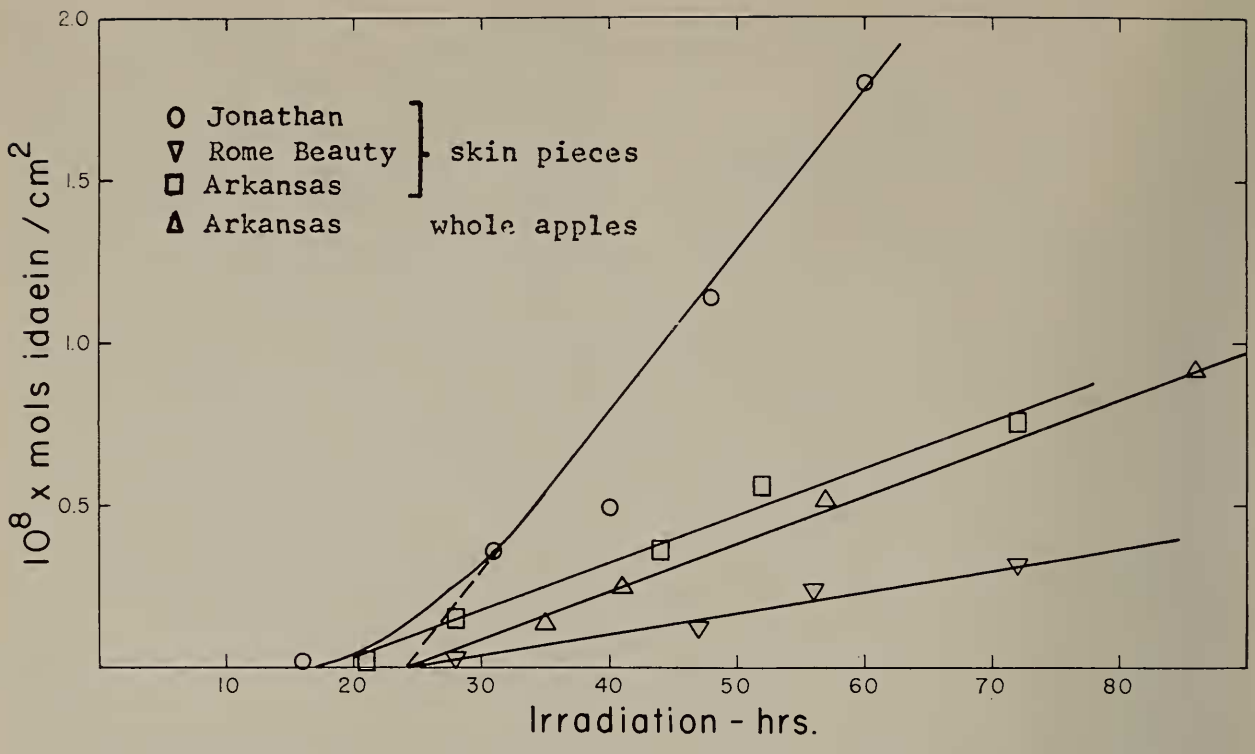

Fig. 4. The dependence of anthocyanin formation in apple skin on the time of exposure under constant irradiance with a fluorescent light source equivalent in photochemical effectiveness to 0.6 milliwatt/cm^2 at 7000 A. Results are shown for skin pieces of Jonathan, Rome Beauty, and Arkansas varieties floating on 0.3 *M* sucrose and for whole Arkansas apples (10).

light conditions. Following the exposure to light, the pieces of apple skin were allowed to incubate 24 hours in darkness. After the dark incubation, five sections of peeling were placed in 5 ml of extracting solution (1 percent HCl in methanol). The solutions were placed at 5° C for 24 hours, then the absorbancy at 530 mμ was measured in a spectrophotometer. Optical density was converted to moles idaein/cm^2 by using a molecular extinction coefficient of 3.43 x 10^4.

The time course of anthocyanin synthesis in apple skin (Fig. 4) shows a nonreciprocal time and intensity relation similar to that found in milo, turnip, and cabbage seedlings. Apples, however, require a greater period of illumination, and the time lag is so great that it may be regarded as a light-requiring preinduction period. During the preinduction period almost no anthocyanin is formed, but the duration of the preinduction period depends on the intensity of the light, the temperature, and the time for equilibration to occur between the appleskin tissue and the sucrose medium. The induction phase of anthocyanin synthesis in apple skin is linear with the duration of the exposure to light as was the case in milo, turnip, and red cabbage seedlings. During the preinduction period for apple and turnip some substrate rises to a level that permits formation of anthocyanin at a rate proportional to the intensity (10). No preinduction period is required in milo and red cabbage, which indicates an adequate level of substrate.

In order for light to produce a physiological response it must be absorbed by some substance within the plant. Photoreceptors generally absorb in specific regions of the spectrum; therefore they can be characterized by their absorption spectra. However, photoreceptors cannot always be isolated for direct absorption measurements, so the absorption characteristics are determined indirectly by the wavelength dependency of the response, that is by an action spectrum. An action

spectrum is determined by placing the biological material in various wavelength regions of the spectrum and discovering how much energy is required at each narrow waveband to produce the physiological response being investigated. It follows that the narrower the waveband the more precise the action spectrum.

Action spectra not only characterize the spectral absorbance of the photoreceptor but they also equate different physiological responses to the same photomechanism. The effective use of action-spectra studies is found in the study of phytochrome. Action spectra showed that such diverse light-controlled responses as germination (13), internode elongation (14), leaf expansion (14), initiation of flowers (15), and pigmentation of the tomato-fruit epidermis (16) were controlled by the same photoreceptor. The absorption peculiarities of phytochrome revealed by the action spectra provided the assay by which phytochrome was subsequently extracted and purified (17).

Since phytochrome controls so many diverse plant responses to light, it seems natural to inquire as to whether phytochrome is also the photoreceptor controlling synthesis of anthocyanin. In order to make such an inquiry we must first understand the characteristics and *modus operandi* of the phytochrome system. Phytochrome exists in two forms; a red-absorbing, inactive P_r form that has a maximum absorption near 660 mμ; and a far red-absorbing, physiologically active P_{fr} form that absorbs near 730 mμ. When irradiated with red, P_r is transformed to P_{fr}, and when P_{fr} is irradiated with far red, it is converted to P_r. P_{fr} also slowly reverts to P_r in darkness. Generally, phytochrome-controlled plant responses require relatively low energies for brief periods of time. When the time required for a phytochrome reaction seems unduly long, it could be a result of a restricted supply of the substrate upon which P_{fr} acts. In that case we find that pulses of red radiant energy as well as continuous light keeps enough P_{fr} present long enough to induce the plant response. The frequency of the pulses must be great enough that excessive dark reversion of P_{fr} to P_r does not occur during the intervening dark periods.

Detailed action spectra for anthocyanin synthesis of milo, red cabbage, and turnip seedlings, and for apple-skin sections were determined with a large prism-type spectrograph (18). All material studied showed that irradiation in the blue region of the spectrum resulted in anthocyanin synthesis. Activity at longer wavelengths, however, varied from none in milo seedlings to maximal activity at 650 mμ in apple skins, at 690 mμ in red cabbage, and at 725 mμ in turnip seedlings. Because of the long-wavelength response, the possible control of anthocyanin synthesis by phytochrome was examined. Red cabbage seedlings, which form some anthocyanin in darkness, were irradiated briefly with red or red imemdiately followed by far red. The red radiant energy induced an increase in anthocyanin content as compared with synthesis in darkness, and the effect of the red was reversed by a subsequent far-red irradiation (Table 3). However, phytochrome was not clearly resolved as the principal photoreceptor or as a secondary control mechanism. Turnip

Table 3. Anthocyanin formation in turnip and red cabbage seedlings irradiated with an energy of about 0.1 joule/cm² of red (580-690 mμ) and/or far red (690-800 mμ) (8)*

Type of irradiation	*Anthocyanin content per seedling*	
	Red cabbage	*Turnip*
	10^{-10} moles	10^{-10} moles
None	25	2.97
Red	38	2.94
Far red	26	2.97
Far red, red	37	—
Red, far red	29	—

* Turnip seedlings were irradiated after induction of anthocyanin synthesis by exposure for 4 hours to a fluorescent source.

seedlings were irradiated for 4 hours to induce anthocyanin formation then irradiated with red or far red, but anthocyanin synthesis was unresponsive to the state of phytochrome.

In milo seedlings anthocyanin is clearly controlled by two photoreactions. The first photoreaction requires high intensities of light and exposures of several hours, and it has a maximum sensitivity near 470 mμ. The second reaction controls the effects of the first one and is a typical phytochrome response. Intensities are low, exposure times are a matter of minutes, and a maximum inhibitory effect is obtained between 710 and 750 mμ. The effects of the far-red irradiation are reversed by a subsequent irradiation in the red region of the spectrum between 630 and 670 mμ (Table 4).

Apple anthocyanin seemed to be unresponsive to the state of phytochrome. However, these early tests were made at the close of the total light period of about 40 hours. More recent investigations have shown a definite phytochrome control of anthocyanin synthesis in apple-skin sections. An inquiry was made into the stability of the products of the preinduction period which seemed to be required for successful operation of the linear induction phase. When various durations of darkness were placed between the preinduction and the induction phases, about 40 percent of the effect of the preinduction period was lost in about 24 hours (Table 5). If the dark period was preceded by a brief irradiation with far red, the loss of preinduction effect was greater. The effect of the far red was reversed when the far red was followed by an exposure to red (Table 6).

The details of the photocontrol of anthocyanin synthesis are confounded by the presence and operation of two photoreceptors; one is unknown and the other is the ubiquitous phytochrome. Siegelman and Hendricks (8) called the first photoreaction the high-energy reaction (HER) because it required more energy than did

Table 4. Reversibility of anthocyanin formation by far-red and red radiant energy (11)*

*Exposures ***		
Far red (number)	*Red (number)*	*Anthocyanin* ($A \times 10^{-2}$)
0	0	106
1	0	48
1	1	106
27	26	45
27	27	109
38	37	48
38	38	97
42	41	49
42	42	103

* After 3-hour exposure to an illuminance of 2,000 ft-c from fluorescent lamps.

** Three minutes of far red; 1 minute of red.

Table 5. Idaein formation in pieces of Arkansas apple skin as affected by a dark interval between the 16-hour preinduction and the 24-hour induction periods

Dark interval (hours)	*Idaein* (10^{-6} *moles/cm²*)
0	6.92
24	4.27
32	4.06
48	1.76
56	1.45

Table 6. Idaein formation in pieces of Arkansas apple skin as affected by the condition of phytochrome at the beginning of a 24-hour dark interval separating the 16-hour preinduction and the 24-hour induction periods

Treatment	*Idaein* (10^{-6} *moles/cm²*)
No dark interval	5.72
24-hr dark interval	3.45
10 min far red, 24-hr dark interval	2.88
10 min far red, 5 min red, 24-hr dark interval	3.58

phytochrome. The name has been perpetuated by Mohr and is involved in other plant responses to light than anthocyanin synthesis (9, 12).

What is the HER and what is the photoreceptor? Photosynthesis is a possibility because it is a high-energy system and is

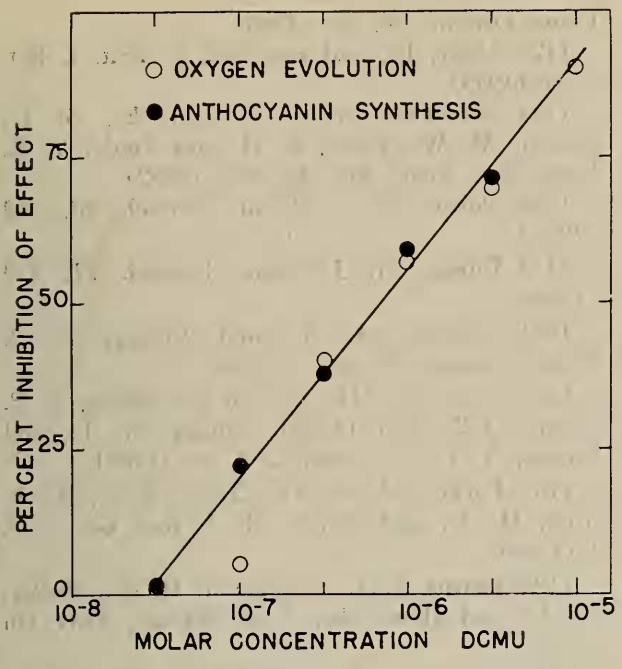

Fig. 5. Inhibition of photosynthesis (oxygen evolution) and anthocyanin synthesis in apple skin by 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

active in the red and blue regions. Moreover, chlorophyll is present in the apple skin and photosynthesis does take place (19). However, the production of soluble carbohydrate by photosynthesis has been ruled out because the young seedlings used for anthocyanin studies are still self-sufficient from cotyledons (12) and endosperm, and sucrose must be added to the medium for apple-skin sections if an appreciable amount of anthocyanin is to be produced. However, the processes of photosynthesis might be required to supply some substrate other than soluble carbohydrate.

Emerson et al. (20) showed that the poor yield of photosynthesis produced by far red was enhanced by supplemental radiation of shorter wavelengths. From this enhancement effect and the subsequent work of Duysens (21) and others, it is generally agreed that electrons are transferred from water to pyridine nucleotide by two chlorophyll systems. Wavelengths in the region of 680 to 730 $m\mu$ are generally more effective in system 1, and action spectra for photosynthesis responses which are closely related to system 1 have maxima in the region. System 2 contains most of the chlorophyll b, and photosynthetic responses which depend on system 2 generally show an action maximum at 650 $m\mu$.

One manifestation of the dual pigment system is the change in fluorescence of chlorophyll that accompanies supplemental radiation in the red and far red. Apple-skin sections show a 30 percent greater fluorescence yield following supplemental red as compared to far-red radiation. Thus, the dual pigment system functions in the apple skin (19).

DCMU (3(3, 4-dichlorophenyl)-1, 1-dimethylurea) inhibits photosynthetic electron transport without interfering with other metabolic reactions. The fluorescence enhancement in the apple skin was inhibited 75 percent by 2 x 10^{-6} M DCMU and a concentration of 1 x 10^{-6} M inhibited oxygen evolution and anthocyanin synthesis by 50 percent (19). The inhibition of oxygen evolution and that of anthocyanin synthesis in the apple skin were the same for a number of DCMU concentrations (Fig. 5).

The action spectrum for anthocyanin formation in the apple skin suggests that chlorophyll system 2 might be contributing to anthocyanin synthesis. Since the carbon substrate for the HER must be supplied by exogenous sucrose, the photosynthetic system is apparently contributing a supply of an oxidant or reductant, or an energy source such as ATP.

The subsequent control by phytochrome indicates that P_{fr} action occurs on some product of the HER and is, therefore, a separate and different photoreaction than the HER.

The HER maximum in the far red that induces anthocyanin formation in other plant tissues (8, 9, 12) resembles the action of photosynthetic pigment system 1. Although data are not available to support the hypothesis that pigment system 1 is the HER for anthocyanin synthesis in these plants, the idea is not incompatible with

the facts, and investigations of this type are currently in progress.

References

(1) Arthur, J. M. Biological effects of radiation, ed. by Duggar, B. M., Vol. 2, pp. 1109-1118, McGraw-Hill Book Co., 1963.

(2) Senebier, J. Physiologie vegetale, Geneva, 1799.

(3) Onslow, M. W. The anthocyanin pigment of plants, 2nd ed., Univ. Press, Cambridge, 1925.

(4) Willstätter, R. Ann. Chem. (Liebig) *401*, 189-232 (1913).

(5) Harborne, J. B. J. Chromatography *1*, 473-488 (1958).

(6) Geissman, T. A., and Jurd, L. Arch. Biochem. Biophysics *56*, 259 (1955).

(7) Acheson, R. M., Harper, J. L., and McNaughton, I. H. Nature *178*, 1283 (1956).

(8) Siegelman, H. W. and Hendricks, S. B. Plant Physiol. *32*, 393 (1957).

(9) Mohr, H. Planta *49*, 389 (1957).

(10) Siegelman, H. W., and Hendricks, S. B. Plant Physiol. *33*, 185 (1958).

(11) Downs, R. J., and Siegelman, H. W. Plant Physiol. *38*, 25 (1963).

(12) Mohr, H., and van Nes, E. Zeit. f. Bot. *51*, 1 (1963).

(13) Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H. and Toole, V. K. Proc. Nat. Acad. Sci. *38*, 662 (1952).

(14) Downs, R. J. Plant Physiol. *30*, 468 (1955).

(15) Downs, R. J. Plant Physiol. *31*, 279 (1956).

(16) Piringer, A. A., and Heinze, P. H. Plant Physiol. *29*, 467 (1954).

(17) Borthwick, H. A., and Hendricks, S. B. Science *132*, 1223 (1960); Butler, W. L., and Downs, R. J. Sci. Amer. *203*, 56 (1960).

(18) Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Scully, N. J. Bot. Gaz. *108*, 1 (1946).

(19) Downs R. J., Siegelman, H. W., Butler, W. L., and Hendricks, S. B. Nature, 1964 (in press).

(20) Emerson, R., Chalmers, R., Cedarstrand, C., and Brody, M. Science *123*, 673 (1956).

(21) Duysens, L. N. M. Proc. Roy. Soc. *B 157*, 301 (1963).

The Teaching Crisis*

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Forty-one years ago this month, Professor Ben Peterson gave me the first teaching assignment in which I had complete charge of the course. This was a class of student nurses who were taking chemistry at night after spending 12 hours mopping floors, emptying bed pans, and doing all the thousand and one chores that were expected of student nurses in those days. How much the nurses learned cannot now be determined, but I learned some chemistry and I learned to love teaching. From that day to this, I have been associated with universities, always either as an active teacher or in administrative work closely allied to teaching. I am now becoming increasingly concerned with the pressures that are continually being put on professors to devote portions of their time, often large portions, to activities other than teaching. These pressures at times cause the professor to neglect his teaching and at other times drive him completely from teaching. Both are events that even rich America cannot afford. It is to this problem that I wish to address myself tonight, and my excuse for taking your time is that the past 41 years have given me some background in this area.

* Address of the retiring president before the Washington Academy of Sciences on February 20, 1964. The opinions expressed in this paper are those of Dean Van Evera alone, and are not necessarily those of either George Washington University or the Washington Academy of Sciences.